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CLINICAL IMMUNOLOGY

PRINCIPLES AND PRACTICE

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discrimination would imply infinite intrinsic affinity (negative free energy change of complex formation), which is not physically plausible.[17] Second, the convexity of atoms prevents perfect shape complementarity between antibody and antigen.[18]

In contrast to the epitope-centered forms of specificity just described, a third aspect of specificity relates to the ability of an antibody to discriminate between antigens that display many copies of one or more distinct epitopes. An antigen expressing many copies of one epitope is termed *multivalent*, and an antigen that expresses two or more different epitopes is referred to as *multideterminant* (Table 14.1). Because two different organisms may both express multiple copies of the same or a similar epitope, an antibody that is highly specific (in the first sense above) for such a shared epitope may be a poor discriminator between such multivalent particles.[19] Yet an antibody with a relatively poor degree of complementarity and intrinsic affinity for a given epitope, found on only one of two or more multivalent targets, may be superior at discriminating between these antigens. Furthermore, antibodies (or other molecules) expressing two or more binding sites with identical structures may not discriminate identically among antigens displaying the same epitope in different two- or three-dimensional distributions.[11]

Some final points regarding specificity: first, for many purposes, immunological specificity has an ultimately biological, not a physical, definition. If the end-point of analysis is the triggering of a complex response, such as cell activation or initiation of the complement cascade, then the presence, absence or extent of that response, and not the extent of receptor–antigen interaction, will be the ultimate criterion for evaluating specificity. Second, the enormous utility of antibodies is crucially dependent on the discriminatory abilities of these molecules with respect to other molecules or molecular aggregates. However, given that the discrimination mediated by antibodies is not absolute, the usefulness of a particular antibody may depend on which antigens or potential antigens are available for binding to the antibody. Third, apparent antibody specificity may vary with the methods used for analysis, as these methods may differ in sensitivity and in environmental conditions (pH, ionic strength, temperature).[20]

Protein epitopes

Several categories of epitope have been defined for protein antigens, based on the proximity of the relevant amino acids in the primary structure of the protein (Fig. 14.3). The simplest case is the *linear* epitope, where all of the amino acids constituting the epitope are derived from a contiguous stretch of the polypeptide chain. However, many – perhaps most – epitopes on globular proteins involve amino acids from two or more stretches of polypeptide that are distant from one another in the primary structure. Such an epitope is referred to as *conformational* or *discontinuous*. In some cases it is conceivable that a conformational epitope can comprise amino acids derived from separate, adjacent polypeptide chains. Another category of protein epitope, the *neo-epitope*, is reserved for those antigenic sites that become recognizable only after a post-translational event, such as proteolytic cleavage. For example, several neo-epitopes have been defined on cleavage products of human C1q, C3 and C9, components of the complement pathway.[21] Antibodies recognizing such neo-epitopes can be used to monitor the extent of activation of the complement pathway.[21]

The first structure of an antibody-variable module in complex with a globular protein antigen, determined by X-ray crystallography,[1] indicated that protein epitopes, defined on the basis of intermolecular contact, could be as large as 15–20 amino acids. A similar number of amino acids in the antibody V domains constituted the paratope. Of course, it is possible that there are smaller epitopes on globular proteins, particularly for regions of proteins that protrude or have a high radius of curvature. A recent crystallographic study suggests that a peptide antigen–antibody interaction can involve as many as 12 peptide amino acids in contact with the antibody.[22]

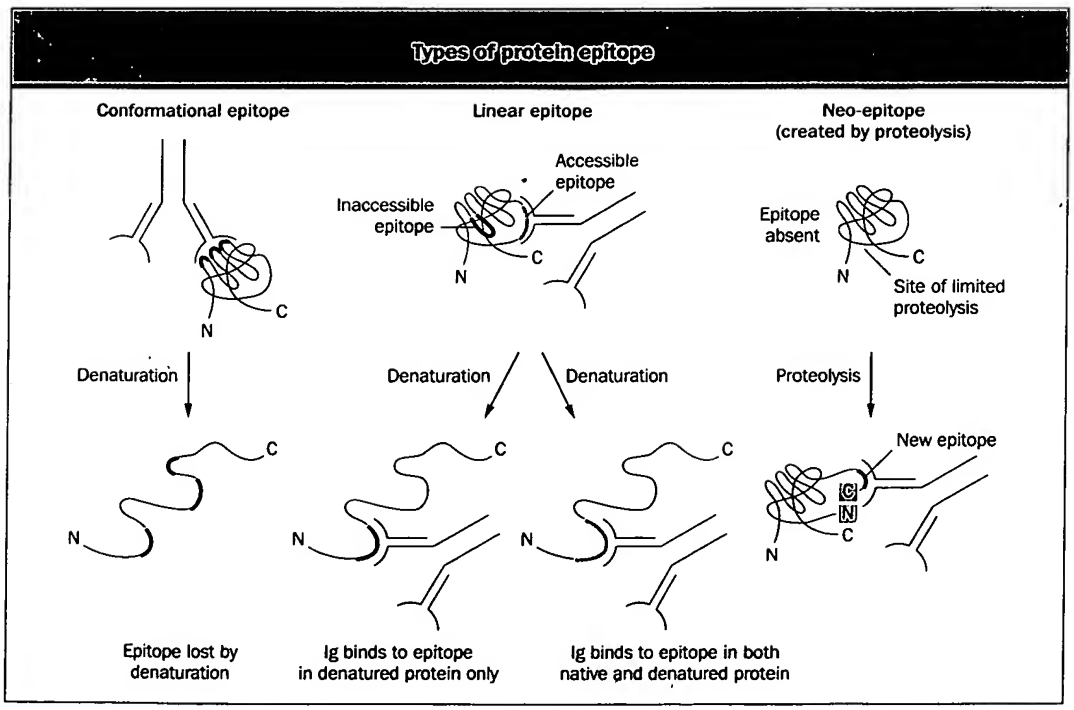

Figure 14.3 Types of protein epitope. Some antibodies recognize structural features of proteins that arise from the folding of the polypeptide backbone (conformational epitope). Other antibodies recognize groups of amino acid residues that are contiguous, or nearly so, in the primary (covalent) structure of the protein (linear epitope). If such a linear determinant is inaccessible in the native structure of the protein, the corresponding antibodies may only be elicited by the denatured form of the protein. Neo-epitopes are created by covalent post-translational modifications, such as proteolytic cleavage. (From Abbas AK, Lichtman AH, Pober JS. *Cellular and molecular immunology*, 3rd edn. WB Saunders 1997, with permission.)